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PP-X. Detection, quantification and differential analysis of the vapour-phase-mediated antimicrobial activity of essential oil volatiles in standard multi-well plates

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Abstract

In the search for antimicrobials, *in vitro* standardized bioassays are indispensable and commonly a first step in a long investigative process. To quantify their antimicrobial potential, the minimal inhibitory concentration, *i.e.* the lowest concentration of a (mixture of) chemical(s) that prevents visible growth of a microorganism, is typically determined using a broth microdilution method. However, some of these antimicrobials have a relatively high vapour pressure at room temperature *e.g.* essential oil components, which may allow them to also exert their antimicrobial activity (AA) over a distance. The AA can be a *direct* result of the vapour-phase of the antimicrobial as quantified with *e.g.* the disc volatilization assay, a method derived from the commonly used agar diffusion assay. However, the AA can also be an indirect result of the vapour-phase of the antimicrobial dissolving at a distance, which we named the vapour-phase-mediated antimicrobial activity (VMAA).

We introduced novel assays to evaluate the VMAA using a standard 96-well microtitre plate and a procedure based on a standardized protocol of a broth microdilution assay. To characterize the assay, we determined the VMAA of a large collection of essential oil(s) (components) and antifungals against two pathogenic human *Candida* species. We showed that there was no correlation between the VMAA and the minimal inhibitory concentration of the essential oil(s) (components), indicating that these are complementary measures. Furthermore, we showed that *C. glabrata* was on average more susceptible to essential oil(s) (components) than *C. albicans* and identified the essential oil(s) (components) with a significantly differential VMAA. As such, this is the first detailed characterization of a novel approach to qualitatively and quantitatively assess the VMAA of molecules using standard multi-well plates.

Keywords: vapour-phase-mediated antimicrobial activity, essential oil, volatility, multi-well plate, *Candida*

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